A phylogeny of the northern temperate leafy liverwort genus *Scapania* (Scapaniaceae, Jungermanniales)

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ABSTRACT

Scapania is a northern temperate genus with a few disjunctions in the south. Despite receiving considerable attention, the supraspecific classification of this genus remains unsatisfactorily solved. We use three molecular markers (nrITS, cpDNA *trn*L-F region, *atp*B-*rbc*L spacer) and 175 accessions belonging to 50 species (plus eight outgroup taxa) to estimate the phylogeny and to test current classification systems. Our data support the classification of *Scapania* into six rather than three subgenera, rearrangements within numerous sections, and inclusion of *Macrodiplophyllum microdontum*. *Scapania* species with a plicate perianth form three early diverging lineages; the most speciose subgenus, *Scapania* s.str., represents a derived clade. Most morphological species concepts are supported by the molecular topologies but classification of sect. *Curtae* requires further study. Southern lineages are nested in northern hemispheric clades. Palearctic–Nearctic distribution ranges are supported for several species.

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1. Introduction

Scapania (Dumort.) Dumort. is a large, terrestrial genus of leafy liverworts with a fossil record that dates back to the Paleogene (Grolle and Schmidt, 2001; Frahm, 2006). Potemkin (2002) accepted 87 species in three subgenera and 18 sections. The genus has its center of diversity in the northern hemisphere, but a few species occur also in the southern hemisphere (Váňa et al., 2009). Besides a few narrow endemics, *Scapania* includes numerous intercontinentally distributed species. A few circumpolar species have remarkable disjunct tropical occurrences (Gradstein and Váňa, 1987; O'Shea et al., 2003; Engel and Glenny, 2008). *Scapania* has not only been studied morphologically but also in terms of secondary metabolite patterns (Asakawa, 2004), and is well known for its metal accumulation capacity (López and Caballeira, 1993; Vincent et al., 2001) and usefulness as bioindicator in freshwater systems (Grasmück et al., 1995).

At the genus level, *Scapania* can normally be identified by the complicate-bilobed, non-vittate leaves with ovate-obovate lobes, having a smaller dorsal than ventral segment, the frequent occurrence of smooth, ovoid to ellipsoidal gemmae developing from the leaf lobes, and the usually smooth, flat perianths. Species taxonomy is regarded as much more difficult, possibly because of the rather monotonous genus morphology and the frequent presence of depauperate or sterile forms that can hardly be identified using morphological evidence (Schuster, 1974; Meinunger and Schröder, 2007). As a consequence, many taxa are still subject to controversy, and different authors arrive at different taxonomic conclusions (Potemkin, 1999a; Damsholt, 2002; Meinunger and Schröder, 2007; Zuo et al., 2007).

Recent molecular phylogenetic studies of liverwort genera are often incongruent with morphology-based supraspecific classifications and have led to adjustments of existing classification schemes (Groth et al., 2004; Heinrichs et al., 2004; Hentschel et al., 2007, 2009). Molecular data have also changed our views about species delineations and species ranges (see Shaw, 2001 and Heinrichs et al., 2009 for reviews). Several molecular phylogenetic studies

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included a few representatives of *Scapania* (e.g., Schill et al., 2004; Yatsentyuk et al., 2004; De Roo et al., 2007) but the limited sampling did not allow for comprehensive taxonomic conclusions. A recent molecular study comprised 37 *Scapania* accessions, mainly from Russia (Vilnet et al., 2010). This study pointed to numerous inconsistencies between existing supraspecific classifications, and molecular topologies.

Here we extend the sampling of Vilnet et al. (2010) and produce the first global phylogeny of *Scapania*, based on 175 accessions belonging to 50 species (plus eight outgroup species) and sequences of the nuclear ribosomal internal transcribed spacer region, the chloroplast DNA intergenic *atp*B-*rbc*L spacer, and the *trn*L-F region. We test the supraspecific classification of Potemkin (2002), and examine the phylogenetic delimitation of species by including multiple accessions from different regions of their ranges. We confirm intercontinental ranges of several morphologically circumscribed species, provide evidence for incongruence of morphological classifications and molecular topologies, and postulate morphologically cryptic speciation in several binomials.

2. Material and methods

2.1. Taxon sampling, distribution ranges and outgroup selection

Taxa studied are listed in Table 1 with GenBank accession numbers and voucher details. All voucher specimens were carefully examined and original identifications were corrected when necessary. *Scapania* taxa were sampled based on Potemkin (2002) to cover the morphological variation of the genus over its geographic range. Most species are represented by multiple accessions. Distribution range information is based on recent floras and checklists (Schuster, 1974; Stotler and Crandall-Stotler, 1977; Paton, 1999; Damsholt, 2002; Potemkin, 2002; Söderström et al., 2002; McCarthy, 2006; Engel and Glenny, 2008; Lai et al., 2008; Konstantinova et al., 2009) and our own observations.

A first dataset was compiled which included each one representative of the investigated *Scapania* species and representatives of the related genera *Diplophyllum* (Dumort.) Dumort., *Douinia* (C.E.O. Jensen) H. Buch and *Macrodiplophyllum* (H. Buch) Perss. (Vilnet et al., 2010). *Lophozia ventricosa* (Dicks.) Dumort. and *Tritomaria quinquedentata* (Huds.) H. Buch were designated as outgroup taxa based on the phylogenies of Schill et al. (2004), Heinrichs et al. (2005a), De Roo et al. (2007) and Vilnet et al. (2009, 2010). The circumscription of *Scapania* was assessed on the basis of this first dataset, and sister lineages to *Scapania* (*Douinia*, *Diplophyllum*, *Macrodiplophyllum* s. str.) designated as outgroups for the extended dataset which includes multiple accessions of *Scapania* species.

2.2. DNA extraction, PCR amplification and sequencing

Plant tissue from the distal portions of a few shoots was isolated from herbarium collections. Total genomic DNA was purified using Invisorb Spin Plant Mini Kit (Invitek, Berlin, Germany) prior to amplification.

Protocols for PCR were carried out as described in previous publications: nrITS region from Feldberg et al. (2004), *trn*L-F region from Feldberg and Heinrichs (2006), and the *atp*B-*rbc*L spacer from Feldberg et al. (2010). Bidirectional sequences were generated using a MegaBACE 1000 automated sequencing machine using DYEnamic ET Primer DNA Sequencing Reagent (Amersham Biosciences, Little Chalfont, UK). Sequencing primers were those used for PCR. Voucher specimens for all sequences were deposited in GOET or JE. Three hundred and ninety-three sequences were newly generated for this study, 94 sequences were downloaded from Gen-

bank. Sequences of the outgroup taxa *Tritomaria quinquedentata* and *Lophozia ventricosa* come from different accessions (Table 1).

2.3. Phylogenetic analyses

All sequences were aligned manually in Bioedit version 7.0.5.2 (Hall, 1999). Ambiguous positions were excluded from the alignment and lacking parts of sequences were coded as missing. Maximum parsimony (MP) analyses were carried out with PAUP* version 4.0b10 (Swofford, 2000). MP heuristic searches of the small and the extended datasets were conducted with the following options implemented: heuristic search mode, 100 random-addition-sequence replicates, tree bisection-reconnection (TBR) branch swapping, MULTrees option on, and collapse zero-length branches off. All characters were treated as equally weighted and unordered. Non-parametric bootstrapping values (Felsenstein, 1985) were generated as heuristic searches with 1000 replicates, each with ten random-addition replicates. The number of rearrangements was restricted to ten millions per replicate (extended dataset, Fig. 2) or unrestricted (small dataset, Fig. 1). Bootstrap percentage values (BPV) ⩾70 were regarded as good support (Hillis and Bull, 1993). Where more than one most parsimonious tree was found, trees were summarized in a strict consensus tree.

The three genomic regions were first analysed separately to check for incongruence. The strict consensus trees of the non-parametric bootstrap analyses were compared by eye to identify conflicting nodes supported by at least 70% (Mason-Gamer and Kellogg, 1996). The trees gave no evidence of incongruence. Hence the datasets were combined.

jModeltest 0.1 (Posada, 2008) was used to select a model of evolution for the maximum likelihood (ML) analyses of the large combined dataset. ML trees were generated using the program GARLI version 0.96 beta (Zwickl, 2006) using the GTR model with Γ-parameter and invariant site variable implemented and all parameters estimated. All analyses were performed with the default settings and several times repeated. The default setting of GARLI was also employed to calculate bootstrap values for ML analyses based on 300 bootstrap replicates.

3. Results

3.1. Small combined dataset

Alignment of the three genomic regions resulted in a combined matrix with the following: *atp*B-*rbc*L 559 positions, *trn*L-F 410 positions, nrITS 817 positions. Of a total of 1786 character sites 1029 were constant, 254 autapomorphic and 503 parsimony informative (Table 2). Maximum parsimony analyses of the combined dataset yielded 24 maximally parsimonious trees of 1976 steps, consistency index (CI) 0.53, and retention index (RI) 0.68. The strict consensus of these trees is depicted in Fig. 1. All clades assigned to subgenera or sections, as well as the backbone, achieve good bootstrap support. Three species of *Diplophyllum* form a clade that is placed sister to the rest of the ingroup. The subsequent clade comprises of *Douinia ovata* (Dicks.) H. Buch, *Macrodiplophyllum imbricatum* (M. Howe) Perss. and *M. plicatum* (Lindb.) Perss. in a well supported relationship with the *Scapania* clade (BPV 88). The *Scapania* clade receives a BPV of 100. *Scapania microdonta* (Mitt.) Müll. Frib. [*Macrodiplophyllum microdontum* (Mitt.) Perss.] forms the sister to all other *Scapania* species. The subsequent clade comprises only *S.* (subg. *Ascapania* Grolle) *contorta* Mitt., followed by a clade assigned to *S.* subg. *Plicatycalyx* Müll. Frib., and a clade corresponding to *S.* sect. *Gracilidae* H. Buch. The Neotropical *S.* (subg. *Macroscapania* R.M. Schust.) *portoricensis* Hampe & Gottsche is placed sister to *S.* subg. *Scapania*. Several morphologically circumscribed sections are polyphyletic. *Scapania*

Table 1

Taxa used in the present study, including information about the origin of the studied material, voucher information, and the herbarium where the voucher is deposited, as well as GenBank accession numbers. Sequences in bold were obtained from GenBank. Herbarium acronyms follow Holmgren et al. (1990).

Taxon	Origin	Voucher and Herbarium	GenBank Acc. Nos.		
			nrITS	trnL-F	atpB-rbcL
Diplophyllum albicans (L.) Dumort.	Germany, Thuringia, Hermsdorf	Hentschel Bryo240 (JE)	–	JN631491	JN631624
Diplophyllum albicans	Norway, Spitsbergen	Konstantinova K 121-6-06 (KPABG)	**EU791774**	**EU791660**	–
Diplophyllum obtusifolium (Hook.) Dumort.	Germany, Bavaria, Teuschnitz	Hentschel Bryo 2592 (GOET)	JN631354	JN631492	JN631625
Diplophyllum obtusifolium	Poland, Malopolska, Szczawnica	Hentschel Bryo 882	JN631355	JN631493	JN631626
Diplophyllum taxifolium (Wahlenb.) Dumort.	Germany, Lower Saxony, Braunlage	Hentschel Bryo 2044 (GOET)	JN631356	JN631494	JN631627
Douinia ovata (Dicks.) H. Buch	Belgium, Hautes Fagnes, Hagelscheid	Keizer s.n. (GOET)	JN631357	JN631495	JN631628
Douinia ovata	Canada, British Columbia, Vancouver	Schofield & Davison 1809 (GOET)	JN631358	JN631496	**AY453600**
Lophozia ventricosa (Dicks.) Dumort.	United Kingdom	Long 31226 (E)	**DQ875101**	**AY453777**	–
Lophozia ventricosa	Russia	Bakalin 11-5-00 (KBAG)	–	–	–
Macrodiplophyllum imbricatum (M. Howe) Perss.	U.S.A., Alaska	Konstantinova 110-2-92 (KPABG)	**EU791770**	**EU791658**	–
Macrodiplophyllum plicatum (Lindb.) Perss.	Russia: Kamchatskaya Prov	Bakalin 22-08-2001 (KPABG)	**EU791768**	**AF519198**	–
Scapania aequiloba (Schwägr.) Dumort.	Austria, Tyrol, Lermos – Biberwier	Düll 14-8-2002.1/14 (JE)	JN631359	JN631497	JN631629
Scapania aequiloba	Germany (I), Thuringia, Großburschla	Marstaller 2-7-2001 (JE)	JN631360	JN631498	JN631630
Scapania aequiloba	Germany (II), Bavaria, Bayreuth Distr.	Meinunger & Schröder 3-5-2000 (JE)	JN631361	JN631499	JN631631
Scapania aequiloba	Czech Republic, Prielom Dunajca, Szczawnica	Hentschel Bryo 863 (GOET)	JN631362	JN631500	JN631632
Scapania aequiloba	Switzerland (I), Niwalden, Wolfenschiessen	Hentschel & Busch Bryo 2826 (GOET)	JN631363	JN631501	JN631633
Scapania aequiloba	Switzerland (II), Graubünden, Engadin	Gradstein 7862 (GOET)	JN631364	JN631502	JN631634
Scapania americana Müll. Frib.	U.S.A. (I), California, San Mateo Co.	Shevock 27851 (GOET)	JN631365	JN631503	JN631635
Scapania americana	U.S.A. (II), California, San Mateo Co.	Shevock 27855 (GOET)	JN631366	JN631504	JN631636
Scapania americana	U.S.A., Oregon, Douglas Co.	Shevock 26373 (GOET)	JN631367	JN631505	–
Scapania americana	U.S.A., Washington	Konstantinova A22-6a-95 (KPABG)	**EU791764**	**EU791655**	JN631637
Scapania ampliata Steph.	Japan (I), Ehime Pref., Mt. Ishizuchi	Higuchi, Bryo. Sel. Exs. 1074 (JE)	JN631368	JN631506	JN631638
Scapania ampliata	Japan (II), Kyushu, Yakoshima Isl.	Yokoyama 11576 (GOET)	JN631369	JN631507	–
Scapania apiculata Spruce	Russia, Siberia, Buryatiya Rep.	Konstantinova HRE 49 (KPABG)	**EU791741**	**EU791633**	**GQ899906**
Scapania aspera M. Bernet & Bernet	Austria, Carinthia, Hermagor	Schäfer-Verwimp & Verwimp 27469 (GOET)	**EU791735**	**EU791627**	JN631639
Scapania aspera	Belgium	Konstantinova 2-20-3-99 (KPABG)	**GQ900012**	**GQ900222**	JN631640
Scapania aspera	Bulgaria, Rhodope Mts., Backovo	Hentschel Bryo 762 (GOET)	JN631370	JN631508	JN631641
Scapania aspera	Germany (I), Saxony-Anhalt, Nebra	Marstaller 13-3-2002 (JE)	JN631371	JN631509	JN631642
Scapania aspera	Germany (II), Baden-Wurttembergia, Sigmaringen	Schäfer-Verwimp & Verwimp 28588 (GOET)	JN631372	JN631510	JN631643
Scapania aspera	Germany (III), Lower Saxony, Bad Sachsa	Heinrichs et al. 3700 (GOET)	JN631373	JN631511	–
Scapania aspera	Germany (IV), North Rhine-Westphalia, Dahlem	Düll 3-12-1994-1 (JE)	JN631374	JN631512	JN631644
Scapania aspera	Germany (V), Thuringia, Kyffhäuser	Marstaller 8-10-2004 (JE)	JN631375	JN631513	JN631645
Scapania aspera	Germany (VI), Thuringia, Nordhausen	Marstaller 26-5-2003 (JE)	JN631376	–	–
Scapania aspera	Germany (VII), Bavaria, Kulmbach	Meinunger 12-4-2001 (JE)	JN631377	JN631514	JN631646
Scapania aspera	Germany (VIII), Baden-Wurttembergia, Hohenstein	Schröder 17-8-1999 (JE)	JN631378	JN631515	JN631647
Scapania aspera	Germany (IX), Baden-Wurttembergia, Tuttlingen	Schäfer-Verwimp & Verwimp 28888 (GOET)	JN631379	JN631516	JN631648
Scapania aspera	Germany (X), Hesse, Eschwege	Marstaller 15-7-2002 (JE)	JN631380	JN631517	JN631649
Scapania aspera	Italy (I), Sicily, Palermo	Düll & Düll 17a.05-06-1990 (JE)	JN631381	JN631518	JN631650
Scapania aspera	Italy (II), The Marches, Fabriano	Marstaller 6-9-2001 (JE)	JN631382	JN631519	JN631651
Scapania bolanderi Austin	Canada, British Columbia (I), Graham Isl.	Hong 98-157 (JE)	JN631383	JN631520	JN631652
Scapania bolanderi	Canada, British Columbia (II), Vancouver Isl.	Priddle s.n. (GOET)	JN631384	JN631521	JN631653
Scapania bolanderi	U.S.A., California (I), San Mateo Co.	Shevock 29906 (GOET)	JN631385	JN631522	–
Scapania bolanderi	U.S.A., California (II), San Mateo Co.	Whittemore 6738 (GOET)	JN631386	JN631523	JN631654
Scapania bolanderi	U.S.A., California (III), San Mateo Co.	Shevock 27778 (GOET)	JN631387	JN631524	JN631655
Scapania bolanderi	U.S.A., Washington (I)	Konstantinova A10-4-95 (KPABG)	**EU791767**	**EU791657**	–
Scapania calcicola (Arnell & J. Perss.) Ingh.	U.S.A., Washington (II), Cowlitz Co.	Kellman & Shevock 5767 (GOET)	JN631388	JN631525	JN631656
Scapania calcicola	Germany (I), Thuringia, Pössneck	Hentschel Bryo 1300 (GOET)	JN631389	JN631526	JN631657
Scapania ciliata Sande Lac.	Germany (II)	Konstantinova 28VIII86 (KPABG)	**EU791757**	**EU791648**	–
Scapania ciliata	China (I), Guangxi, Laibin	Ye & Wei 20090719-10 (HSNU)	JN631390	JN631527	JN631658
Scapania ciliata	China (II), Guizhou, Yuao	Peng 20100518-13 (HSNU)	JN631391	JN631528	JN631659
Scapania ciliata	Japan, Shikoku, Mt. Kokuzo	Deguchi 32735 (GOET)	JN631392	JN631529	JN631660
Scapania ciliata	Nepal, Kangchenjunga, Sikdim – Chauki	Long 17560 (JE)	JN631393	JN631530	JN631661

Table 1 (continued)

Taxon	Origin	Voucher and Herbarium	Genbank Acc. Nos.		
			nrITS	trnL-F	atpB-rbcL
Scapania ciliatospinosa Horik.	Bhutan, Tongsa, Yuto La	Long 8038 (JE)	JN631394	JN631531	JN631662
Scapania ciliatospinosa	Nepal (I), Upper Tadi Khola	Miehe & Miehe 15830 (JE)	JN631395	–	–
Scapania ciliatospinosa	Nepal (II), Kangchenunga, Nesum-Buje Daurali	Long 17508 (JE)	JN631396	JN631532	–
Scapania ciliatospinosa	Nepal (III), Langtang	Miehe & Miehe 16481 (JE)	JN631397	JN631533	–
Scapania compacta (Roth) Dumort.	Germany, Saxony Anhalt, Treseburg-Thale	Eckstein 1409 (GOET)	JN631398	JN631534	JN631663
Scapania compacta	Italy (I), Sardinia, Monte Limbara	Marstaller 16-9-99 (JE)	–	JN631535	JN631664
Scapania compacta	Italy (II), Liguria, Cinque Terre	Marstaller 20-9-2002 (JE)	–	JN631536	JN631665
Scapania compacta	Spain, La Palma, Cubo de Galga	Huneck JE-H3294 (JE)	JN631399	JN631537	JN631666
Scapania compacta	United Kingdom, Argyll, Glencoe	Long & Murray 11492 (JE)	JN631400	JN631538	JN631667
Scapania contorta Mitt.	Nepal, Kangchenjunga, Ghunsa	Long 16930 (JE)	JN631401	JN631539	JN631668
Scapania crassiretis Bryn	Russia (I), Murmansk	Konstantinova 354-5b-00 (KPABG)	**EU791755**	**EU791646**	–
Scapania crassiretis	Russia (II), Murmansk, Kola Peninsula	Huneck 1 (JE)	JN631402	JN631540	JN631669
Scapania curta (Mart.) Dumort.	Czech Republic, Ústecký kray, Marienberg	Eckstein 3896 (GOET)	JN631403	JN631541	JN631670
Scapania curta	Germany (I), Saxony-Anhalt, Breitenbach	Hentschel Bryo 3174 (GOET)	JN631404	JN631542	JN631671
Scapania curta	Germany (II), Bavaria, Jehsen	Schröder 20-5-2002 (GOET)	JN631405	JN631543	JN631672
Scapania curta	Poland, Beskid Sadecki-Pasmo Radziejowey, Szczawnica	Hentschel Bryo 0874 (GOET)	JN631406	JN631544	JN631673
Scapania curta (cf.)	U.S.A., California, El Dorado Co.	Whittemore 417B (GOET)	JN631407	JN631545	JN631674
Scapania cuspiduligera (Nees) Müll. Frib.	Austria, Tyrol, Pfunds	Gradstein 7828 (GOET)	JN631408	JN631546	JN631675
Scapania cuspiduligera	Russia, Buryatia	Konstantinova 24-1-02 (KPABG)	**EU791752**	**EU791643**	–
Scapania ferruginea (Lehm. & Lindenb.) Gottsche	Bhutan, Thimphu Distr., Motithang	Long 10838 (JE)	JN631409	JN631547	JN631676
Scapania ferruginea	India, Sikkim	Long 22492 (KPABG)	**EU791766**	**AF5119193**	–
Scapania ferruginea	Nepal (I), S of Langtang Khola	Poelt H3065 (JE)	JN631410	JN631548	JN631677
Scapania ferruginea	Nepal (II), Ghunsa Khola, Amjilassa – Kyapra	Long 16650 (JE)	JN631411	JN631549	JN631678
Scapania ferruginea	Nepal (III), Ghunsa	Long 16928 (JE)	JN631412	JN631550	JN631679
Scapania ferruginea	Nepal (IV), S of Langtang Khola	Poelt H3066 (JE)	JN631413	JN631551	–
Scapania ferruginea	Nepal (V), Ghunsa – Tamo La	Long 16953 (JE)	JN631414	JN631552	JN631680
Scapania ferruginea	Nepal (VI), Kangchenjunga, Tseram	Long 17126 (JE)	JN631415	JN631553	JN631681
Scapania glaucocephala (Tayl.) Austin	Russia, Siberia, Buryatiya Rep.	Konstantinova 64-05-02 (KPABG)	**EU791644**	**EU791644**	–
Scapania gracilis Lindb.	Portugal, Azores	Schäfer-Verwimp & Verwimp SV29543 (GOET)	JN631416	JN631554	JN631682
Scapania gracilis	Portugal, Madeira, Seixal	Schäfer-Verwimp & Verwimp 25636 (GOET)	JN631417	JN631555	JN631683
Scapania gracilis	United Kingdom, South Devon, Dartmoor	Eckstein & Esser 6245 (GOET)	JN631418	JN631556	JN631684
Scapania griffithii Schiffn.	China (I), Fujian, Dehua Co.	Zhu et al. 20100403-21 (HSNU)	JN631419	JN631557	JN631685
Scapania griffithii	China (II), Fujian, Dehua Co.	Zhu et al. 20100403-25 (HSNU)	JN631420	JN631558	JN631686
Scapania griffithii	Nepal, Kangchenjunga, Sikdim-Chauki	Long 17552 (JE)	JN631421	–	JN631687
Scapania gymnostomophila Kaal.	Germany (I), Thuringia, Nordhausen	Marstaller 25-5-2005 (JE)	JN631422	JN631559	JN631688
Scapania gymnostomophila	Germany (II), Thuringia, Rüdigsdorf	Marstaller 6-11-2003 (JE)	JN631423	JN631560	–
Scapania gymnostomophila	Russia, Murmansk	Konstantinova 13-1-98 (KPABG)	**EU791758**	**EU791649**	–
Scapania hedbergii S.W. Arnell	Kenya	Chuah-Petiot 588 (JE)	JN631424	–	–
Scapania helvetica Gottsche	Russia (I), Caucasus, Karachayevo-Cherkessian Rep.	Konstantinova K414-1-05 (KPABG)	**EU791728**	**EU791620**	–
Scapania helvetica	Russia (II), Caucasus, Karachayevo-Cherkessian Rep.	Konstantinova 488-3-05 (KPABG)	**EU791727**	**EU791619**	–
Scapania hyperborea Jørg	Norway, Aust-Agder, Haukeligrend	Hentschel Bryo 3230 (GOET)	JN631425	JN631561	JN631689
Scapania hyperborea	Russia, Murmansk	Konstantinova 509-3a-04 (KPABG)	**EU791744**	**EU791635**	–
Scapania hyperborea	Russia, Yakutia	Bakalin 1-10-00 (KPABG)	**EU791745**	**EU791636**	–
Scapania irrigua (Nees) Nees	Germany (I), Thuringia, Piesau	Meinunger 3-8-2002 (JE)	JN631426	JN631562	–
Scapania irrigua	Germany (II), Lower Saxony, Alfeld	Schröder 5-10-1995 (JE)	JN631427	JN631563	JN631690
Scapania irrigua	Germany (III), Thuringia, Lobenstein	Meinunger 14-9-2002 (JE)	JN631428	JN631564	–
Scapania irrigua	Germany (IV), Bavaria, Lusen	Schröder 3-6-1996 (JE)	JN631429	JN631565	JN631691
Scapania irrigua	Germany (V), Bavaria, Ilz valley	Hentschel & Busch Bryo 3168 (GOET)	JN631430	JN631566	JN631692
Scapania irrigua	Germany (VI), Saxony-Anhalt, Breitenbach	Hentschel Bryo 3277 (GOET)	JN631431	JN631567	JN631693
Scapania irrigua	Norway, Aust-Agder, Haukeligrend	Eckstein 42 (GOET)	JN631432	JN631568	JN631694
Scapania irrigua	Poland (I), Beskid Sadecki, Obidza	Hentschel Bryo 876 (GOET)	JN631433	JN631569	JN631695
Scapania irrigua	Poland (II), Beskid Sadecki, Szczawnica	Konstantinova 18-1-02 (KPABG)	JN631434	JN631570	JN631696
Scapania irrigua	Russia, Buryatiya	Konstantinova 18-1-02 (KPABG)	**EU791729**	**EU791621**	–
Scapania irrigua	Russia, Komi Rep.	Dulin 118-99 (KPABG)	**EU791731**	**EU791623**	–

Taxon	Voucher	Locality			
Scapania irrigua	Konstantinova K469-1-04 (KPABG)	Russia, Maryi-El Rep.	**EU791730**	**EU791622**	–
Scapania irrigua	Konstantinova 219-4-02 (KPABG)	Russia, Murmansk	**EU791733**	**EU791625**	–
Scapania irrigua	Konstantinova K 372-5-04 (KPABG)	Russia, Permskiy Kray	**EU791732**	**EU791624**	JN631697
Scapania javanica Gottsche	Gradstein 12046 (GOET)	Indonesia, Central Sulawesi, Mt. Rorekatimbu	JN631435	JN631571	JN631698
Scapania javanica	Schäfer-Verwimp & Verwimp 24861 (GOET)	Indonesia, Sumatra, Berastagi	JN631436	JN631572	–
Scapania kaurinii Ryan	Bakalin 11-1-00 (KPABG)	Russia, Chita Prov.	**EU791759**	**EU791650**	JN631699
Scapania koponenii Potemkin	Zhu et al. 20090630-48 (HSNU)	China (I), Zhejiang, Suichang Co.	JN631437	JN631573	JN631700
Scapania koponenii	Zhu et al. 20090630-22 (HSNU)	China (II), Zhejiang, Suichang Co.	JN631438	JN631574	JN631701
Scapania lepida Mitt.	Eggers SL 5.01 (JE)	Sri Lanka, Central Prov., Nuwara Eliya	JN631439	JN631575	JN631702
Scapania ligulata Steph.	Wei 20090705124 (HSNU)	China (I), Guangxi, Maozhou	JN631440	JN631576	JN631703
Scapania ligulata	Zhu et al. 20100406-1B (HSNU)	China (II), Fujian, Dehua Co.	JN631441	JN631577	JN631704
Scapania ligulata	Long 17499 (JE)	Nepal, Kangchenjunga, Nesum-Buje Daurali	JN631442	JN631578	JN631705
Scapania ligulata	Huneck KDVR 88-36 (JE)	North Korea, Kumgangsan, Manmulsang	JN631443	JN631579	JN631706
Scapania lingulata H. Buch	Marstaller 22-2-99 (JE)	Germany, Thuringia, Eisenach	JN631444	–	JN631707
Scapania microdonta (Mitt.) Müll. Frib.	Bakalin P-74-11-05 (GOET)	Russia, Far East, Primorye Terr.	JN631445	JN631580	JN631708
Scapania microdonta	Konstantinova 146-12-01 (KPABG)	Russia, Siberia (I), Buryatiya Rep.	**EU791769**	AF519199	–
Scapania microdonta	Konstantinova, Hep. Ross. Exs. 11 (GOET)	Russia, Siberia (II), Buryatiya Rep.	JN631446	JN631581	JN631709
Scapania mucronata	Meinunger & Schröder 19876 (JE)	Germany (I), Bavaria, Hof	JN631447	JN631582	JN631710
Scapania mucronata	Eckstein 4335 (GOET)	Germany (II), Thuringia, Nordhausen	JN631448	JN631583	JN631711
Scapania mucronata	Bakalin 43-8-01 (KPABG)	Russia, Karelia	**EU791738**	**EU791630**	JN631712
Scapania mucronata	Bakalin 100854 (KPABG)	Russia, Tuva Rep.	**EU791737**	**EU791629**	JN631713
Scapania nemorea (L.) Grolle	Konstantinova 1-20-9-99 (KPABG)	Belgium	**EU791754**	**EU791645**	JN631714
Scapania nemorea	Schäfer-Verwimp & Verwimp 28792 (GOET)	Germany (I), Bavaria, Lindau	JN631449	JN631584	JN631715
Scapania nemorea	Heinrichs & Schmidt 3732 (GOET)	Germany (II), Hesse, Vockerode	JN631450	JN631585	JN631716
Scapania nemorea	Hentschel Bryo 931 (GOET)	Germany (III), Thuringia, Hermsdorf	JN631451	JN631586	JN631717
Scapania nemorea	Heinrichs 294 (GOET)	Luxemburg, Berdorf	JN631452	JN631587	JN631718
Scapania nemorea	Schäfer-Verwimp & Verwimp 25744 (GOET)	Portugal, Madeira, Curral Falso – Fanal	JN631453	JN631588	JN631719
Scapania nemorea	Allen 22667 (GOET)	U.S.A., Maine (I), Franklin Co.	JN631454	JN631589	AY453605
Scapania nemorea	Allen 25916 (GOET)	U.S.A., Maine (II), Oxford Co.	JN631455	JN631590	JN631720
Scapania nimbosa Taylor ex Lehm.	Long 35929 (E)	China, Yunnan		JN631591	JN631721
Scapania nimbosa	Long 38039 (E)	United Kingdom, Scotland	JN631456	JN631592	–
Scapania obcordata (Berggr.) S.W. Arnell	Konstantinova 123-1-04 (KPABG)	Norway (I), Spitsbergen	**EU791734**	**EU791626**	JN631722
Scapania obcordata	Hentschel Bryo 0389 (GOET)	Norway (II), Spitsbergen, Nordenskiöld Land	JN631457	JN631593	JN631723
Scapania ornithopodioides (With.) Waddell	Long 16447 (JE)	Nepal, Kangchenjunga, Basantpur - Door Pani	JN631458	JN631594	JN631724
Scapania ornithopodioides	Long 29820 (E)	United Kingdom, Scotland, Argyll	**EU791743**	AY453782	JN631725
Scapania paludicola Loeske & Müll. Frib.	Bakalin 11VIII1997 (KPABG)	Russia, Karelia	JN631459	AF519196	JN631726
Scapania paludicola	Long et al. 40399 (E)	United Kingdom, Wales, Glamorgan	JN631460	JN631595	JN631727
Scapania paludosa (Müll. Frib.) Müll. Frib.	Faubert & Grenier 232 (GOET)	Canada, Quebec, Gaspé Penins.	JN631461		–
Scapania paludosa	Schäfer-Verwimp 19613 (GOET)	Germany, Baden-Wurttembergia, Furtwangen	**EU791747**	JN631596	JN631728
Scapania paludosa	Konstantinova 4-3-00 (GOET)	Russia, Kemerovo	**EU791748**	**EU791638**	JN631729
Scapania paludosa	Konstantinova K316-2-04 (KPABG)	Russia, Permskiy Kray	JN631462	**EU791639**	JN631730
Scapania portoricensis Hampe & Gottsche	Schäfer-Verwimp & Verwimp 14737 (GOET)	Brazil, Minas Gerais, Serra da Mantiqueira	JN631463	JN631597	–
Scapania portoricensis	Churchill et al. 22023 (GOET)	Bolivia (I), Santa Cruz, Manuel M. Caballero	JN631464	JN631598	–
Scapania portoricensis	Churchill et al. 20651 (GOET)	Bolivia (II), Santa Cruz, Manuel M. Caballero	JN631465	JN631599	–
Scapania portoricensis	Dauphin 1246 (GOET)	Costa Rica, Chirripó	JN631466	JN631600	–
Scapania portoricensis	Churchill et al. 24297 (GOET)	Ecuador, Zamora-Chinchipe, El Pangui	JN631467	JN631601	–
Scapania portoricensis	Allen 14279 (GOET)	Honduras, Cortes, Cusuco N.P.	JN631468	JN631602	–
Scapania rotundifolia W.E. Nicholson	Long 16977 (JE)	Nepal, Kangchenjunga, Tamo La – Sinion La	**EU791746**		–
Scapania rufidula Warnst.	Bakalin 35-3-00 (GOET)	Russia, Yakutia	JN631469	**EU791637**	–
Scapania scandica (S.W. Arnell & H. Buch) Macvicar	Meinunger 20420 (JE)	Germany, Bavaria, Anberger Forst	**EU791762**	JN631603	–
Scapania simmonsii Bryn & Kaal.	Konstantinova 45-9-98 (KPABG)	Russia, Murmansk	JN631470	**EU791653**	–
Scapania simmonsii	Schofield 77966 (GOET)	U.S.A., Alaska, Sukakpak Mt.	JN631471	JN631604	–
Scapania sphaerifera (H. Buch) Tuom.	Konstantinova, Hep. Ross. Exs. 20 (JE)	Russia (I), Siberia, Buryatiya Rep.	**EU791765**	JN631605	–
Scapania sphaerifera	Konstantinova 92-2-01 (KPABG)	Russia (II), Siberia, Buryatiya Rep.	**EU791761**	**EU791656**	–
Scapania spitsbergensis (Lindb.) Müll. Frib.	Konstantinova 90-2-06 (KPABG)	Norway, Spitsbergen	**EU791760**	**EU791652**	–
Scapania spitsbergensis	Konstantinova 121-6-02 (KPABG)	Russia, Buryatiya Rep.	JN631472	**EU791751**	–
Scapania subalpina (Lindenb.) Dumort.	Urbaniak s.n. (JE)	Poland, Easter Carpathians, Zywiec Beskids	JN631473	JN631606	–
Scapania subalpina	Huneck 28-6-1990 (JE)	Russia, Murmansk (I), Kola Penins.	JN631474	JN631607	JN631731
Scapania subalpina	Konstantinova, Hep. Ross. Exs. 50 (GOET)	Russia, Murmansk (II), Khibiny Mts.		JN631608	JN631732

Table 1 (continued)

Taxon	Origin	Voucher and Herbarium	Genbank Acc. Nos.		
			nrITS	trnL-F	atpB-rbcL
Scapania subalpina	U.S.A., Colorado, Summit Co.	Weber & Miller 1.3 (GOET)	JN631475	JN631609	JN631733
Scapania subnimbosa Steph.	China, Fujian, Dehua Co.	Zhu et al. 20100403-131 (HSNU)	JN631476	JN631610	JN631734
Scapania subnimbosa	China, Guangxi, Maozhou	Wei 20090707389 (HSNU)	JN631477	JN631611	JN631735
Scapania subnimbosa	China, Zhejiang, Suichang Co.	Zhu et al. 20090629-116 (HSNU)	JN631478	JN631612	JN631736
Scapania tundrae (Arnell) H. Buch	Norway, Spitsbergen	Konstantinova 140-1-04 (KPABG)	**EU791725 & EU791742**	**EU791634**	–
Scapania uliginosa (Lindenb.) Dumort.	Austria (I), Tyrol, Kühtai	Schäfer-Verwimp & Verwimp 18181 (GOET)	JN631479	JN631613	JN631737
Scapania uliginosa	Austria (II), Tyrol, Serfaus	Gradstein 7769 (GOET)	JN631480	JN631614	–
Scapania uliginosa	Russia, Murmansk	Bakalin 25-7-01 (KPABG)	**EU791739**	**EU791631**	JN631738
Scapania umbrosa (Schrad.) Dumort.	Germany (I), Lower Saxony, Goslar	Eckstein 6509 (GOET)	JN631481	JN631615	–
Scapania umbrosa	Germany (II), Bavaria, Steinberg	Schröder 31-5-1996 (JE)	JN631482	JN631616	JN631739
Scapania umbrosa	Germany (II), Bavaria, Hirschgrund	Schröder 3-9-1996 (JE)	JN631483	JN631617	JN631740
Scapania undulata (L.) Dumort.	Russia, Komi Rep.	Dulin MD139-1-99 (KPABG)	**EU791740**	**EU791632**	JN631741
Scapania undulata	Italy, Udine, Nassfeld pass	Schäfer-Verwimp & Verwimp 27551 (GOET)	JN631484	JN631618	JN631742
Scapania undulata	Portugal, Madeira (I), Porto Moniz	Schäfer-Verwimp & Verwimp 25913 (GOET)	JN631485	JN631619	JN631743
Scapania undulata	Portugal, Madeira (II), Pico Ruivo	Schäfer-Verwimp & Verwimp 25719 (GOET)	JN631486	JN631620	–
Scapania undulata	Portugal, Madaira (III), Rico Ruivo	Schäfer-Verwimp & Verwimp 25725 (GOET)	JN631487	JN631621	JN631744
Scapania undulata	Russia, Murmansk (I)	Konstantinova 208-2-02 (KPABG)	**EU791751**	**EU791642**	JN631745
Scapania undulata	Russia, Murmansk (II), Khibiny Mts.	Konstantinova, Bryo. Ross. Exs. 21 (GOET)	JN631488	JN631622	JN631746
Scapania undulata	U.S.A., California, Mariposa Co.	Shevock et al. 29009 (GOET)	JN631489	JN631623	–
Scapania verrucosa Heeg	Bhutan, Thimphu, Taba	Long 7842 (JE)	JN631490	–	–
Scapania verrucosa	Russia, Caucasus, Karachayevo-Cherkessian Rep.	Konstantinova 609/6-05 (KPABG)	**EU791763**	**EU791654**	–
Tritomaria quinquedentata (Huds.) H. Buch	Russia	Bakalin s.n. (KBAG)	**EU791804**	**AY463592**	–
Tritomaria quinquedentata	Finland	He-Nygrén & Piippo 1474 (H)	–	–	–
Tritomaria quinquedentata	United Kingdom	Long 29130 (E)	–	–	**AY453601**

irrigua (Nees) Nees (type of sect. *Irriguae* H. Buch) is nested in sect. *Curtae* H. Buch; other *Irriguae* elements form a separate lineage. Several Asian species that are currently assigned to sect. *Nemorosae* H. Buch are placed in another lineage, *S.* sect. *Stephania* Potemkin. *Scapania* (sect. *Ciliatae* Grolle) *spitsbergensis* (Lindb.) Müll. Frib. is nested in sect. *Compactae* H. Buch.

3.2. Large combined dataset

Dataset 2 comprised an *atp*B-*rbc*L, *trn*L-F and an nrITS1-5.8S-ITS2 alignment with 1790 putatively homologous sites (Table 2) and 184 accessions (Table 1). The ML topology (ln = −16,759.6443, Fig. 2) resembles the MP topology found with the small dataset (Fig. 1). Many species with multiple accessions form monophyletic lineages including examples with Palearctic-Nearctic ranges. *Scapania irrigua* is paraphyletic with *S. curta* (Mart.) Dumort., *S. helvetica* Gottsche, *S. obcordata* (Berggr.) S.W. Arnell, and *S. scandica* (S.W. Arnell & H. Buch) Macvicar nested in it. Accessions of *S. ligulata* Steph. form two deep clades.

4. Discussion

4.1. Supraspecific classification and evolution of Scapania

4.1.1. Genus circumscription

Two contradictory hypotheses about the evolution of *Scapania* have been put forward based on morphology. Many authors considered *Scapania* to be a derived genus that evolved from a *Lophozia*-like ancestor with an inflated, plicate perianth and an indefinite leaf keel (e.g., Buch, 1928; Schuster, 1951). In contrast, Potemkin (1998, 1999b) proposed that *Scapania* represents the "basal" genus of Scapaniaceae, from which *Diplophyllum* and *Douinia* were derived via *Lophozia* (Dumort.) Dumort. and *Anastrophyllum* (Spruce) Steph. The latter hypothesis was already contradicted by several earlier molecular phylogenetic studies (Schill et al., 2004; Yatsentyuk et al., 2004; Heinrichs et al., 2005a; Vilnet et al., 2010) and also by Potemkin (2002). The extended sampling of the present study allows further insights into the evolution of *Scapania*. *Scapania* species [including *Scapania* (*Macrodiplophyllum*) *microdonta*] with a plicate perianth are found in early diverging lineages (Fig. 1), providing evidence that the flat, smooth perianth of most extant *Scapania* species represents the apomorphic condition, and that *Scapania* derived from a "lophozioid" ancestor. However, the derived species *S. calcicola* (Arnell & J. Perss.) Ingham, *S. compacta* (Roth) Dumort., *S. gymnostomophila* Kaal. and *S. sphaerifera* (H. Buch) Tuom. also have a plicate perianth mouth, possibly in consequence of reversals to the plesiomorphic condition.

The three species of *Macrodiplophyllum* are placed in two independent lineages. The "*Scapania*-like" (Potemkin, 2002: 332) *Macrodiplophyllum microdontum* is placed sister to the remainder of *Scapania*, and is here treated as an element of *Scapania*. The "*Diplophyllum*-like" (Potemkin, 2002: 332) species *Macrodiplophyllum imbricatum* and the generitype *M. plicatum* (Grolle, 1983) form a sister relationship with *Douinia* (Fig. 1). Based on the topology shown in Fig. 1, *Macrodiplophyllum* s. str. could be treated as an independent genus or, alternatively, could be lowered to a synonym of *Douinia*. A further possible solution for the morphological and molecular incongruences would be an incorporation of *Douinia* and *Macrodiplophyllum* s. str. in *Scapania* (Vilnet et al., 2010). However, *Douinia* differs from all related genera by the presence of unispiral elaters and the lack of gemmae (Buch, 1928; Schuster, 1974; Paton, 1999); *Macrodiplophyllum* s. str. has been aligned with *Diplophyllum* rather than with *Scapania* (Buch, 1928; Schuster, 1974). We propose to keep the three genera as separate entities because the morphological overlap of *Macrodiplophyllum* s. str. and
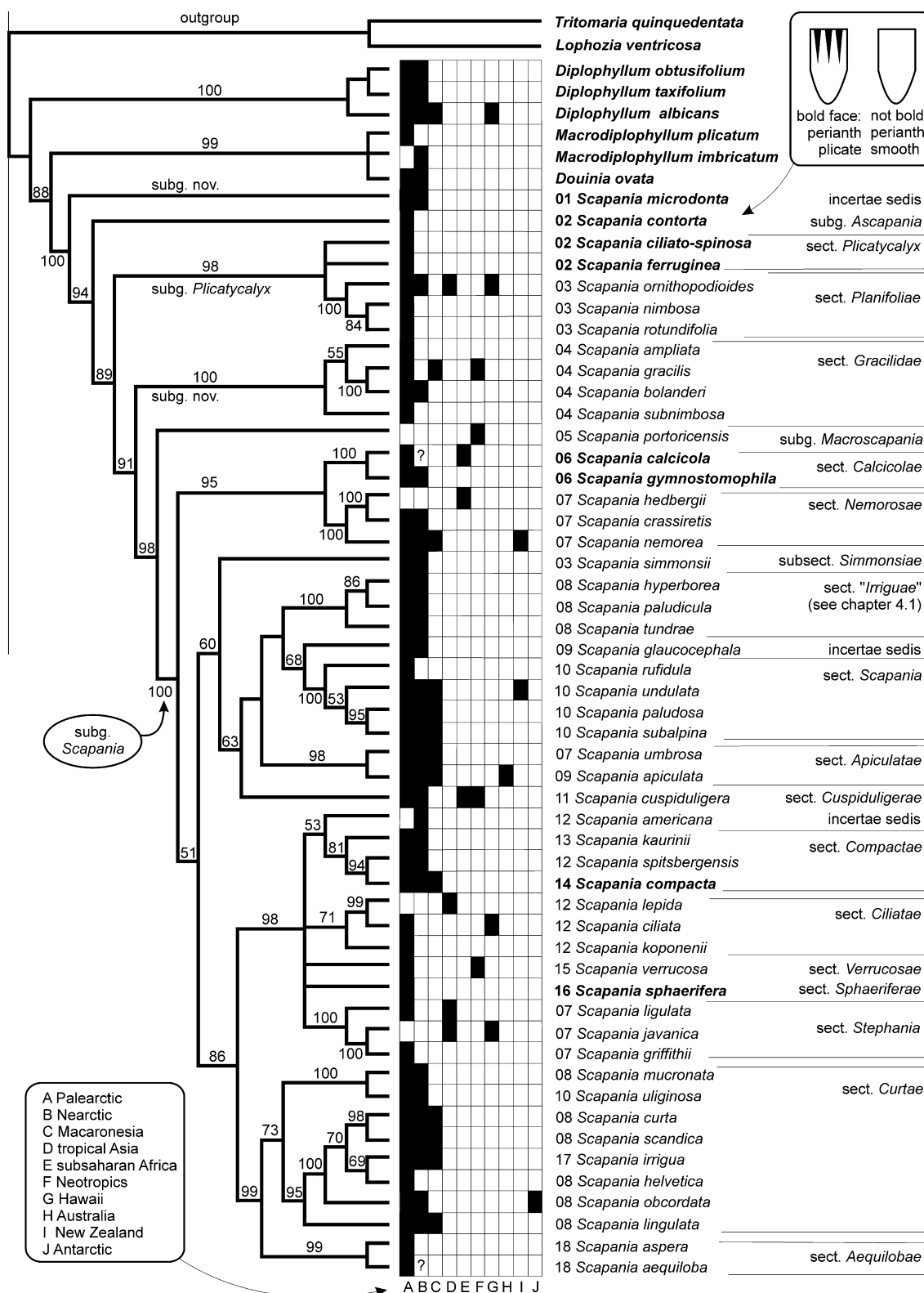
Fig. 1. Strict consensus of 24 equally parsimonious trees (consistency index 0.53) based on an alignment of the nrITS region and the chloroplast DNA markers *trn*L-F and *atp*B-*rbc*L. Distribution of species is indicated at branches. Numbers previous to species names refer to the *Scapania* classification of Potemkin (2002): 01 subg. *Macrodiplophyllum*, 02 sect. *Plicaticalyx*, 03 sect. *Planifoliae*, 04 sect. *Gracilidae*, 05 subg. *Macroscapania*, 06 sect. *Calcicolae*, 07 sect. *Nemorosae*, 08 sect. *Curtae*, 09 sect. *Apiculatae*, 10 sect. *Scapania*, 11 sect. *Cuspiduligerae*, 12 sect. *Ciliatae*, 13 sect. *Incurvae*, 14 sect. *Compactae*, 15 sect. *Verrucosae*, 16 sect. *Sphaeriferae*, 17 sect. *Irriguae*, 18 sect. *Aequilobae*.

Diplophyllum would complicate a morphological circumscription of an extended genus *Scapania*. *Scapaniella* H. Buch was erected for tiny *Scapania* species without distinct stem cortex differentiation and has been treated as a separate genus by Crandall-Stotler et al. (2009). However, the *Scapaniella* elements *Scapania glaucocephala* (Taylor) Austin and *S. apiculata* Spruce are placed in derived lineages of *Scapania*. We therefore consider *Scapaniella* to be a synonym of *Scapania*.

Table 2
Distribution of constant and phylogenetically informative sites for aligned positions of the three genomic regions [(I) small dataset (II) extended dataset)].

	*atp*B–*rbc*L	*trn*L-F	ITS1-5.8S-ITS2	Total
(I) Number of sites in matrix	559	410	817	1786
Constant	332	279	418	1029
Autapomorphic	96	42	116	254
Parsimony informative	131	89	283	503
(II) Number of sites in matrix	559	414	817	1790
Constant	331	272	358	961
Autapomorphic	49	21	84	154
Parsimony informative	179	121	375	675

4.1.2. Classification of Scapania

Potemkin (2002) classified *Scapania* into three subgenera and 18 sections. In this connection he suggested that the sections of *Scapania* are not very sharply defined because of morphological homoplasy. Difficulties in establishing a supraspecific classification of *Scapania* based on morphology are reflected in the conflicting treatments of Müller (1905), Schuster (1974) and Potemkin (1998, 2002), and are also evident from the phylogeny presented in Fig. 1. The monophyly of some of Potemkin's (2002) sections is confirmed (*S.* sects. *Aequilobae* H. Buch, *Calcicolae* R.M. Schust., *Gracilidae*), but others proved to be poly- or paraphyletic. We propose a series of modifications of the species assignments of Potemkin (2002) to render monophyletic supraspecific taxa. However, our reclassification of *Scapania* should be regarded as provisional because we were not able to include all relevant section or subgenus types (still lacking: *S. brevicaulis* Taylor, *S. karl-muelleri* Grolle, *S. secunda* Steph.). The sectional affiliation of a few species (*S. americana* Müll. Frib., *S. glaucocephala*, *S. simmonsii* Bryn & Kaal.) remains unclear based on the present sampling; their classification requires inclusion of further species in the molecular data sets. Several well-supported lineages lack Linnean names; they are outlined in the following two sections and will be formalized elsewhere.

4.1.2.1. Subgenera.
Our topology (Fig. 1) allows for a hierarchical classification of *Scapania* into subgenera and sections. Potemkin (2002) accepted three subgenera, *Macrodiplophyllum*, *Macroscapania*, and *Scapania* s. str. This classification is only partly supported by the present study.

Our treatment of *S.* subg. *Macrodiplophyllum* [s. str.] as a separate genus requires the establishment of a subgenus for the remaining element *Scapania microdonta* (*Macrodiplophyllum microdontum*), which is placed sister to the rest of *Scapania*. This species can be separated from other *Scapania* species by the presence of multicellular gemmae with intersecting walls, in combination with a pluriplicate perianth and basal leaf cell walls with intermediate thickenings. The morphologically similar *S. sphaerifera* (Potemkin, 1999b) is not closely related according to our topologies (Figs. 1 and 2).

The subsequent *Scapania* clade is also made up of a single species, *S. contorta*. *Scapania contorta* represents a peculiar element of *Scapania* that stands out in terms of arcuately inserted dorsal leaf lobes, narrow ventral lobes, brown marginal leaf teeth and pyriform, plicate perianths. Grolle (1966) set up the monospecific subgenus *Ascapania* to accomodate *S. contorta*. The latter subgenus was lowered to a synonym of *S.* sect. *Plicatycalyx* (Müll. Frib.) Potemkin by Potemkin (2002); however, our results do not support this synonymy and we therefore recognize subg. *Ascapania*.

The Neotropical subg. *Macroscapania* includes a few species with long elaters and polygonal gemmae. This subgenus is deeply nested in *Scapania* and is placed sister to a clade including the generitype *Scapania undulata* (L.) Dumort. We assign the latter clade to *S.* subg. *Scapania*. Although we need to exclude several ele-

ments which were aligned with subg. *Scapania* by Potemkin (2002), namely sects. *Plicatycalyx*, *Planifoliae* (Müll. Frib.) Potemkin, and *Gracilidae*, subg. *Scapania* still represents the most speciose subgenus and includes more than 70% of the species diversity. This diverse array of species is difficult to circumscribe morphologically, but includes species that have smooth (rarely weakly plicate), compressed perianths with an entire or toothed mouth, ovoid gemmae, uniseriate antheridial stalks, and moderately elongate elaters.

Our new circumscription of subg. *Scapania* requires the reinstatement of *S.* subg. *Plicatycalyx*. This subgenus represents an early diverging lineage of *Scapania* that comprises sects. *Plicaticalyx* and *Planifoliae*, and includes plants with a (usually) vestigial leaf keel and plicate or nonplicate, dorsiventrally compressed perianths. Maintenance of a classification into subgenera and sections requires furthermore the reassessment of sect. *Gracilidae* to subgenus level. Species of sect. *Gracilidae* are characterized by flat, smooth perianths with a ciliately toothed mouth, dentate dorsal leaf bases and usually greenish gemmae.

In summary, we propose to classify *Scapania* into six subgenera, of which one still needs to be formally described. The early diverging subgenus lineages contain only a few species, in contrast to the derived subgenus *Scapania* which accommodates the majority of extant sections and species.

4.1.2.2. Sections.
Sectional classification of *Scapania* is possibly even more difficult than subgenus circumscription. Some of the entities delimited in Fig. 1 correspond to geographic regions or ecological preferences rather than morphological features, others can be identified by certain combinations of morphological character states. A comprehensive sectional reclassification of *Scapania* will only be possible with a molecular sampling that covers the complete species diversity; morphological homoplasy likely leads to unnatural supraspecific entities if not corroborated by molecular data. Our partial reclassification should thus be regarded as preliminary.

Despite the shortcomings of our limited sampling, we can propose several changes to the current sectional classification systems. Potemkin (2002) regarded sect. *Curtae* as representing a derived group of small-sized species with reduced paraphyses and sparse branching. This view is confirmed by our analyses. However, the *Curtae*-lineage also includes the type of *S.* sect. *Irriguae* (*S. irrigua*), necessitating a synonymization of *Irriguae* and *Curtae* (Vilnet et al., 2010). Other *Irriguae* elements [*S. hyperborea* Jørg., *S. paludicola* Loeske & Müll. Frib., *S. tundrae* (Arnell) H. Buch] are placed in another main clade of subg. *Scapania*, and form a highly supported monophyletic lineage (Figs. 1 and 2a). These plants are usually more robust than *S. irrigua*, and have brownish to reddish gemmae. A sectional name for this clade is currently not available and will be established elsewhere.

Potemkin's (2002) approach to unite morphologically similar species in a single section is sensible but our topology (Fig. 1) indicates parallel evolution of similar morphotypes in different parts of the geographic range of *Scapania*. Several Asian elements of sect. *Nemorosae* sensu Potemkin (2002) form a separate lineage. We therefore propose to reinstate the name *S.* sect. *Stephania* for this clade with a center of diversity in temperate and tropical Asia. *Scapania umbrosa* (Schrad.) Dumort. likewise needs to be excluded from sect. *Nemorosae*, and is here placed in sect. *Apiculatae*. The two representatives of this section (*S. apiculata*, *S. umbrosa*) share a small size, well-defined stem cortex, gradually sharp-pointed leaf lobes, brownish to reddish gemmae, leaf-like female bracts and flattened perianths with an entire mouth.

The two species *S. compacta* and *S. kaurinii* Ryan have been placed in sect. *Compactae* because of their subequally bilobed, entire to remotely dentate, rounded to obtuse leaves with a keel that
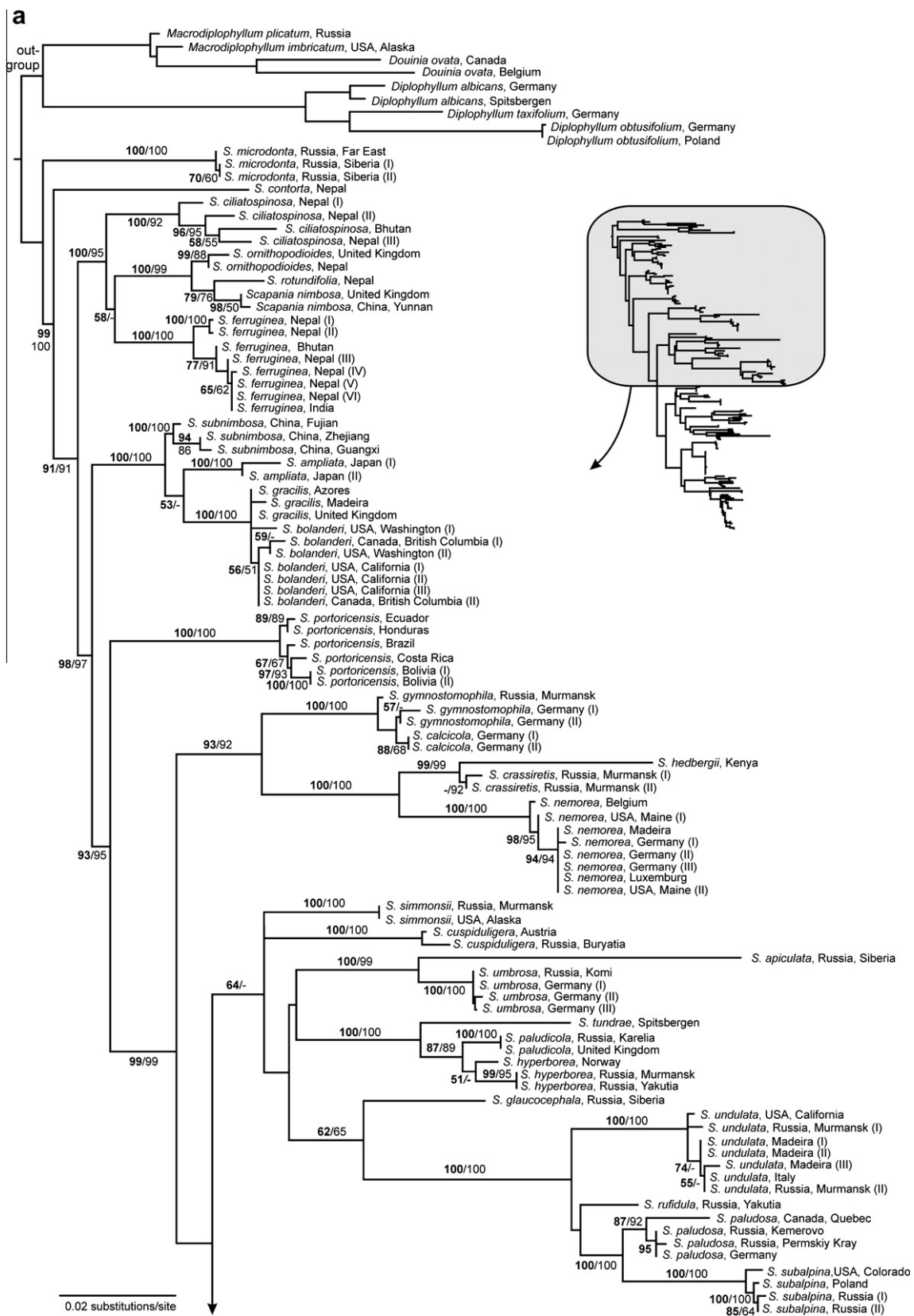
Fig. 2. Phylogram generated in a maximum likelihood analysis of the combined dataset. Bootstrap values are indicated at branches (bold: ML, not bold: MP).

arches sharply away from the stem, and their monoecious sexual condition (Schuster, 1974). In our study, *S. spitsbergensis* proved to be a third species of sect. *Compactae*. This species has been placed in sect. *Ciliatae* by Potemkin (2002) because of its ciliate leaf margin; however, it is a third monoecious *Scapania* species (Schuster, 1974). *Scapania* species are usually dioicious, hence, monoecious condition of species in sect. *Compactae* may be a synapomorphy.
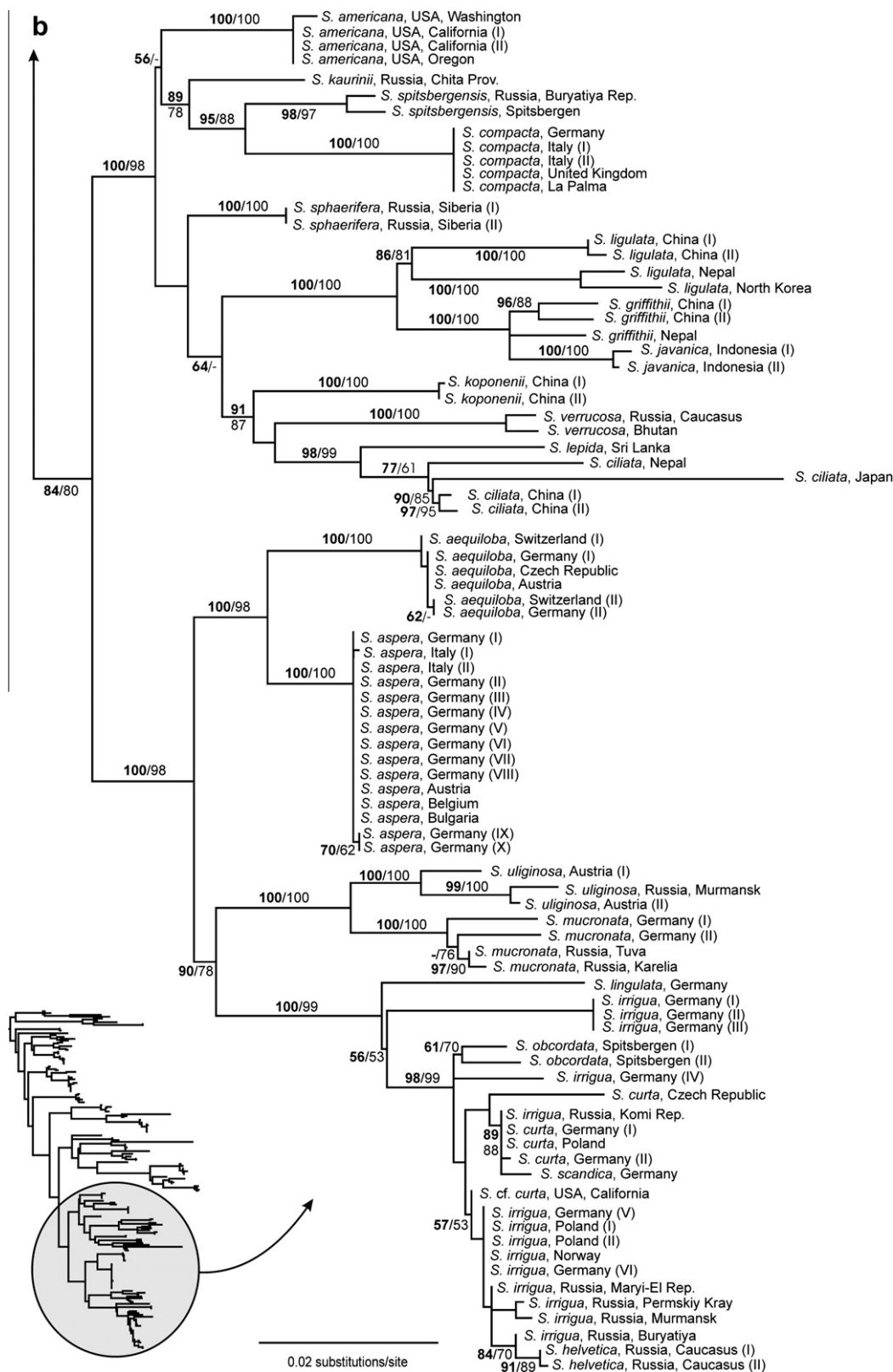
b

100/100 — *S. americana*, USA, Washington
S. americana, USA, California (I)
S. americana, USA, California (II)
S. americana, USA, Oregon
56/-
89/78 — *S. kaurinii*, Russia, Chita Prov.
95/88 — 98/97 *S. spitsbergensis*, Russia, Buryatiya Rep.
S. spitsbergensis, Spitsbergen
100/100 — *S. compacta*, Germany
S. compacta, Italy (I)
S. compacta, Italy (II)
S. compacta, United Kingdom
S. compacta, La Palma
100/98
100/100 — *S. sphaerifera*, Russia, Siberia (I)
S. sphaerifera, Russia, Siberia (II)
86/81 100/100 *S. ligulata*, China (I)
S. ligulata, China (II)
100/100 *S. ligulata*, Nepal
S. ligulata, North Korea
96/88 — *S. griffithii*, China (I)
100/100 100/100 *S. griffithii*, China (II)
S. griffithii, Nepal
100/100 *S. javanica*, Indonesia (I)
S. javanica, Indonesia (II)
64/-
91/87 100/100 *S. koponenii*, China (I)
S. koponenii, China (II)
100/100 *S. verrucosa*, Russia, Caucasus
S. verrucosa, Bhutan
98/99 *S. lepida*, Sri Lanka
77/61 *S. ciliata*, Nepal
S. ciliata, Japan
90/85 *S. ciliata*, China (I)
97/95 *S. ciliata*, China (II)
84/80
100/100 *S. aequiloba*, Switzerland (I)
S. aequiloba, Germany (I)
S. aequiloba, Czech Republic
S. aequiloba, Austria
62/- *S. aequiloba*, Switzerland (II)
100/98 *S. aequiloba*, Germany (II)
100/100 *S. aspera*, Germany (I)
S. aspera, Italy (I)
S. aspera, Italy (II)
S. aspera, Germany (II)
S. aspera, Germany (III)
S. aspera, Germany (IV)
S. aspera, Germany (V)
S. aspera, Germany (VI)
S. aspera, Germany (VII)
S. aspera, Germany (VIII)
100/98 *S. aspera*, Austria
S. aspera, Belgium
S. aspera, Bulgaria
70/62 *S. aspera*, Germany (IX)
S. aspera, Germany (X)
100/100 *S. uliginosa*, Austria (I)
99/100 *S. uliginosa*, Russia, Murmansk
S. uliginosa, Austria (II)
100/100 100/100 *S. mucronata*, Germany (I)
S. mucronata, Germany (II)
-/76 *S. mucronata*, Russia, Tuva
97/90 *S. mucronata*, Russia, Karelia
90/78 100/99 *S. lingulata*, Germany
S. irrigua, Germany (I)
S. irrigua, Germany (II)
S. irrigua, Germany (III)
56/53 61/70 *S. obcordata*, Spitsbergen (I)
S. obcordata, Spitsbergen (II)
98/99 *S. irrigua*, Germany (IV)
S. curta, Czech Republic
S. irrigua, Russia, Komi Rep.
89/88 *S. curta*, Germany (I)
S. curta, Poland
S. curta, Germany (II)
S. scandica, Germany
S. cf. curta, USA, California
57/53 *S. irrigua*, Germany (V)
S. irrigua, Poland (I)
S. irrigua, Poland (II)
S. irrigua, Norway
S. irrigua, Germany (VI)
S. irrigua, Russia, Maryi-El Rep.
S. irrigua, Russia, Permskiy Kray
S. irrigua, Russia, Murmansk
S. irrigua, Russia, Buryatiya
84/70 *S. helvetica*, Russia, Caucasus (I)
91/89 *S. helvetica*, Russia, Caucasus (II)

0.02 substitutions/site

Fig. 2 (*continued*)

Scapania simmonsii was assigned to both sect. *Nemorosae* (Potemkin, 1998) and *Planifoliae* (Potemkin, 2002). Neither of these assignments is confirmed in our study which lends some support to Schuster's (1974) treatment of *S. simmonsii* in a monospecific subsect. *Simmonsiae* R.M. Schust. based on the strongly deflexed-involute ventral leaf lobes and the very large trigones. However,

Schuster's (l. c.) placement of subsect. *Simmonsiae* in sect. *Aequilobae* is not supported. Instead, our data support sectional rank for subsect. *Simmonsiae*.

Potemkin's (2002) concept of sect. *Scapania* is largely confirmed in our study. This section includes hygro- or hydrophytic, large-sized species with entire or shortly toothed, ventrally long

decurrent leaves with broadly rounded lobes and a smooth to weakly papillose cuticle, as well as small trigones. However, our study corroborates the observation of Vilnet et al. (2010) of a close relationship of S. (sect. *Scapania*) *paludosa* (Müll. Frib.) Müll. Frib. and S. (sect. *Curtae*) *mucronata* H. Buch. This relationship was unexpected from morphological and ecological considerations, and deserves further study.

Likewise the separation of the early diverging *Scapania* sections *Plicatycalyx* and *Planifoliae* needs to be further investigated by inclusion of other putatively related species such as S. *karl-muelleri* and S. *orientalis* Müll. Frib. In the ML analysis sect. *Planifoliae* was nested in *Plicatycalyx* albeit without reliable bootstrap support (Fig. 2a). Species of sect. *Plicatycalyx* have plicate perianths, whereas members of sect. *Planifoliae* are provided with flat, smooth ones.

Our study provides evidence that leaf dentation in *Scapania* is of limited taxonomic value above the species level, similar to the situation in the leafy liverwort *Plagiochila* (Dumort.) Dumort. (Heinrichs et al., 2005c). Other gametophytic features of *Scapania* such as leaf and perianth shape, trigone and papillae development or color of gemmae are highly homoplastic as well. In consequence, supraspecific taxa can often only be defined by a certain combination of morphological character states, distribution ranges, and ecological preferences. Species with aberrant morphology relative to their phylogenetic placement complicate diagnosing groups within *Scapania*. Such a situation is not unknown in leafy liverworts, and has also been demonstrated for *Frullania* Raddi (Hentschel et al., 2009), *Lophozia* (Vilnet et al. (2008), *Plagiochila* (Heinrichs et al., 2005b) and *Syzygiella* Spruce (Feldberg et al., 2010). Sporophytic characters such as capsule wall development, spore surface and elater shape may provide additional support for supraspecific entities. They have proven to be variable in *Scapania* (Schuster, 1974; Srivastava and Srivastava, 1994; Potemkin, 2002). However, the amount of available data is presently too incomplete to evaluate the taxonomic importance of the diploid generation of *Scapania*.

4.2. Species taxonomy

Considering the difficulties in recognizing supraspecific entities, many morphological species concepts in *Scapania* work surprisingly well. Inclusion of multiple accessions of many species led to robust monophyletic lineages for the majority of them (Fig. 2); only a few proved to be para- or polyphyletic. The taxonomically most problematic assemblage of species is found in sect. *Curtae* (Fig. 2b). Species identification within this evolutionary young lineage (Potemkin, 2002) is notoriously difficult and requires optimally developed, fertile material, ideally including information on oil bodies that are best seen in living plants (see e.g., the detailed treatments and comments in Schuster, 1974; Paton, 1999; Damsholt, 2002; Meinunger and Schröder, 2007). *Scapania irrigua* proved to be paraphyletic during the course of our study, with accessions of S. *curta*, S. *helvetica*, S. *obcordata*, and S. *scandica* nested within it. The predominantly short branches within the *Curtae* crown group point to recent diversification events that are possibly still imperfectly reflected in morphology. A S. *irrigua* clade with typical, robust phenotypes (accessions Germany I–III) may represent an unrecognized, morphologically cryptic species that deserves further attention. However, deeper insights into the population structure of S. sect. *Curtae* requires a considerable extension of the population sampling, and inclusion of fertile DNA vouchers from which oil body characters have been investigated.

Similarly problematic is the classification of the Asian S. sect. *Stephania* (Fig. 2b). Potemkin et al. (2004) studied the morphology of S. *ligulata* in detail and recognized two morphologically weakly separated subspecies. Our data suggest the presence of two genetically distinct entities; however, the evaluation of putative morphological discrepancies requires a more comprehensive sampling.

Scapania griffithii Schiffn. differs from S. *ligulata* by the frequent presence of leaf lobes with horn-like tips (Potemkin, 2002). Potemkin et al. (2004) doubted the occurrence of S. *griffithii* outside the Himalayan region and adjacent territories. However, we recognized two specimens with the morphology of S. *griffithii* from Fujian, China. In our phylogeny, they form a polytomy with S. *griffithii* from Nepal, and S. *javanica* Gottsche. Extension of the geographical sampling and inclusion of further putatively related taxa such as S. *parvitexta* Steph. and S. *angusta* Müll. Frib. is necessary to gain deeper insights into the taxonomic structure of sect. *Stephania*.

Our study confirms the separation of the Asian S. *koponenii* Potemkin from the European S. *aspera* M. Bernet & Bernet (Potemkin, 2000), which are placed in different main clades of *Scapania*. The considerable sequence differences of S. *nemorea* (L.) Grolle and S. *crassiretis* Bryn support species rather than subspecies rank for the latter taxon (Potemkin, 1994). The African S. *hedbergii* S.W. Arnell is closely related to the Holarctic S. *crassiretis*; however, this observation should be verified with additional accessions. Zehr (1980) reduced S. *paludosa* to a synonym of S. *uliginosa* (Lindenb.) Dumort. This treatment was accepted by Potemkin (1999a, 2002) but rejected by Paton (1999) and Damsholt (2002). It is also not supported in our study with the species in different major clades of *Scapania*. *Scapania calcicola* is nested in the paraphyletic S. *gymnostomophila* Kaal.; however, the Genbank sequences related to the Russian accession should be verified. A close relationship is also indicated between the morphologically similar S. *gracilis* Lindb. and S. *bolanderi* Austin. These species seem to be of recent origin, and can be separated by the slightly different lobe shape and dentition.

4.3. Biogeography

4.3.1. Origin and diversification of Scapania

The distribution patterns mapped onto the tree in Fig. 1 illustrate the Holarctic distribution of extant *Scapania* with a center of diversity in temperate regions of the Palearctic. A combination of molecular and fossil evidence indicates an origin of *Scapania* in the Eocene (Heinrichs et al., 2007), at a time with a globally warm climate, and subtropical forests in large parts of the Northern regions (Willis and McElwain, 2001). It is not unlikely that the first *Scapania* lineages occurred in northernmost regions, and that the cooling processes since the Oligocene (Zachos et al., 2001) led to diversification and colonization into the expanding temperate regions. A similar scenario has recently been proposed for the cool-temperate moss genus *Sphagnum* L. (Shaw et al., 2010), which dominates the cryptogamic vegetation of boreal peatlands.

Many early diverging species of *Scapania* nowadays occur in temperate Asia, and it is not unlikely that the ancestors of most extant *Scapania* lineages come from this region. The investigated southern lineages of *Scapania*, notably S. *portoricensis* and S. *hedbergii*, are nested in northern clades, indicating that the ancestors of these clades occurred in the Holarctic. The Neotropical S. *portoricensis* is an isolated element according to the ML phylogeny (Fig. 2a) and it has no close relatives in the North, providing some evidence for a long period of isolation. Unfortunately we were not able to include the other purely Neotropical *Scapania*, S. *geppii* Steph., in the molecular study. Tropical *Scapania* lineages were obviously not very successful in terms of speciation, in contrast to the temperate elements (or their have been significant differences in extinction patterns). The Paleotropics are likewise poor in *Scapania* species, and harbor a few species such as S. *javanica* and S. *lepida* Mitt. These species are closely related to derived temperate Asian lineages (Fig. 2b), and are thus likely of a more recent origin.

4.3.2. Species ranges

Species of *Scapania* frequently produce gemmae, and are thus good dispersers even in regions with a climate that is unfavorable

for the formation of sporophytes. This ability to reproduce vegetatively by gemmae may explain the success of *Scapania* in the northern regions.

Many morphologically circumscribed *Scapania* species cover both parts of the Holarctic or show other remarkable disjunctions. Molecular studies pointed to a complex genetical structure of many of such circumscribed disjunct bryophytes, and to the presence of numerous morphologically cryptic species (Shaw, 2001; Heinrichs et al., 2009). On the other hand, some disjunct Palearctic-Nearctic liverwort ranges have been confirmed by molecular data, e.g., for the liverworts *Metzgeria pubescens* (Schrank) Raddi (Fuselier et al., 2011), *Frullania tamarisci* (L.) Dumort. (Heinrichs et al., 2010) and *Porella cordaeana* (Huebener) Moore (Heinrichs et al., 2011).

Morphological concepts within *Scapania* are in good accordance with the molecular topology shown in Fig. 2, and Palearctic-Nearctic disjunctions are confirmed for *S. nemorea*, *S. paludosa*, *S. subalpina* (Lindenb.) Dumort. and *S. undulata*. *Scapania nimbosa* Taylor ex Lehm. is a disjunct species with a few stands in the British Isles and Norway, in addition to occurrences in the Sino-Himalaya (Jordal and Hassel, 2010). This remarkable disjunction is confirmed in our study, in which accessions from Scotland and Yunnan form a monophyletic lineage. Similar disjunctions have recently been shown for the liverworts *Anastrophyllum alpinum* Steph. and *A. joergensenii* Schiffn. (Long et al., 2006). It is still unclear if the disjunct range of *S. nimbosa* is the result of extinction (Damsholt, 2002) or expansion processes. Jordal and Hassel (2010) point out that *S. nimbosa* has not yet reached all available localities in southwestern Norway, providing some evidence for ongoing distributional changes. A similar disjunction of *S. ornithopodioides* (With.) Waddell is also confirmed in our study.

Many species of the northern regions of the Holarctic show a bottleneck pattern of genetic diversity, likely in consequence of extinction/expansion processes caused by climate changes of the Pleistocene (Hewitt, 1996, 2000). This pattern is also evident from our ML phylogeny (Fig. 2), however, follow-up studies utilizing hypervariable markers such as microsatellites (Ramaiya et al., 2010) are needed to gain deeper insights into the population structure of these species and their range formation.

4.4. Perspectives

The present study is the first comprehensive phylogeny of *Scapania*, and allows for several adjustments of current classification systems. However, nearly 50 currently accepted species have not yet been included in molecular investigations. Adding these to the present sampling will allow for a comprehensive reclassification of *Scapania*, and a detailed evaluation of morphological character state evolution. *Scapania* sect. *Curtae* is in need of a thorough revision utilizing morphological plus molecular evidence, as are several Asian species complexes. Establishing a stable *Scapania* taxonomy based on an integrative, morphological-molecular approach is desirable to understand the taxonomic positions of numerous forms and varieties that have been established for morphologically aberrant phenotypes. *Scapania* is notorious for such aberrant forms and it is currently unclear if they represent habitat-induced modifications or genetically separated biological entities that deserve formal classification. A reliable classification is also necessary for barcoding *Scapania*. Such a molecular barcode will allow identification of the numerous suboptimally developed phenotypes.

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